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Nitrogen and water requirements of C₃ plants grown at glacial to present carbon dioxide concentrations

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Summary

1. Nitrogen- and water-use efficiencies in biomass production were determined for three C₃ plant species at carbon dioxide concentrations ([CO₂]) that spanned glacial to present atmospheric levels [200–350 µmol CO₂ (mol air)⁻¹]. The species were annual grasses *Bromus tectorum* and *Triticum aestivum* (two cultivars) and a woody perennial *Prosopis glandulosa* (alone and in mixtures with the C₄ grass, *Schizachyrium scoparium*).
2. Changes in nitrogen- and water-use efficiencies were used to investigate effects of increasing [CO₂] on the relative requirements of C₃ plants for these frequently limiting resources.
3. Water-use efficiency (biomass produced/evapotranspiration; WUE) increased at higher [CO₂] in all species but relative responses to [CO₂] varied among species, cultivars and watering regimes.
4. Intrinsic WUE (net assimilation/stomatal conductance to water), calculated from stable carbon isotopes in plants, increased by about the same relative amount as did [CO₂] in all species.
5. Nitrogen-use efficiency (biomass produced/plant N; NUE) rose at higher [CO₂] only in well-watered *B. tectorum* and in *P. glandulosa* grown alone.
6. The more consistent increase in WUE than NUE in these species at higher [CO₂] implies that rising [CO₂] may have reduced the amount of water relative to nitrogen that some C₃ plants require and thereby altered the composition and function of terrestrial ecosystems.

Key-words: *Bromus tectorum*, nitrogen-use efficiency, *Prosopis glandulosa*, stable carbon isotopes, *Triticum aestivum*, water-use efficiency

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Introduction

The concentration of carbon dioxide in the atmosphere ([CO₂]) has increased 25–30% from the pre-industrial level of 270–280 µmol CO₂ (mol air)⁻¹ (Neftel *et al.* 1985; Raynaud & Barnola 1985) and perhaps 100% from a minimum 18 000 years ago, 160–200 µmol mol⁻¹, which is among the lowest in geological history (Delmas, Ascencio & Legrand 1980; Barnola *et al.* 1987). Carbon dioxide may rise to twice the present level during the next century (Trabalka *et al.* 1986).

Most C₃ plants grow more rapidly when [CO₂] is increased (reviews by Bazzaz 1990; Poorter 1993), especially when environmental conditions are benign and resources that plants consume and require for

growth are readily available (Bazzaz 1990). All C₃ plants require the same resources and, over the long term, must acquire different resources in rather fixed proportions to grow (Bloom, Chapin & Mooney 1985; Chapin *et al.* 1987). Consequently, plant growth and its response to additional CO₂ is retarded when the balance of resource availabilities deviates greatly from that required (Field *et al.* 1992).

Annual availabilities of most plant resources, with the possible exception of water, vary little at a given location. So, unless plants are able to gain more of the resources that limit their growth from an essentially fixed total pool, their maximum growth response to higher [CO₂] is largely set by the efficiency with which limiting resources are used in production.

Higher [CO₂] reduces the amounts of some resources that plants must expend to acquire carbon (C) and thereby increases the efficiency with which

these resources are used in plant growth. Influences of higher [CO₂] on the efficiency of utilization of plant resources vary, however, with the nature of the resource. Most of acquired water and intercepted light, for example, are consumed in processes related to C acquisition. At a given [CO₂], the efficiencies with which plants use water and light are largely independent of the rate at which they are supplied from the environment and the amount of each that is consumed. In contrast, essential mineral elements like nitrogen (N) must be retained at a minimum concentration in all plant tissues, including those not directly involved in C uptake. This requirement places an upper limit on plant N-use efficiency (NUE), biomass produced per unit N and may make NUE sensitive to N availability.

Relatively little is understood of how past changes in atmospheric [CO₂] affected growth (Baker, Allen & Boote 1990; Allen *et al.* 1991; Polley, Johnson & Mayeux 1992a) and resource requirements of C₃ plants (Polley *et al.* 1993a). The amount by which increasing [CO₂] stimulated plant growth, however, depended on the identities and relative availabilities of limiting resources and on the efficiencies with which these resources were used in plant production. We use published data of water-use efficiency (WUE) and NUE for one C₃ species, the woody legume *Prosopis glandulosa* Torr. (mesquite), and new data for two C₃ annual grasses, *Bromus tectorum* L. (downy chess or cheatgrass) and *Triticum aestivum* L. (wheat), to investigate how the glacial to present increase in [CO₂] may have altered the relative requirements of C₃ plants for these frequently limiting resources. We then discuss what a change in plant requirement for water relative to N (ratio of NUE to WUE) implies about the response of C₃ plants to the doubling of atmospheric [CO₂] since glaciation.

Materials and methods

CONTROLLED ENVIRONMENT CHAMBER

Results are taken from three experiments in which C₃ plants were grown over a gradient of daytime [CO₂] from near 350 to 200 µmol mol⁻¹. *Bromus tectorum*, *P. glandulosa* and *T. aestivum* were grown in a ventilated glasshouse within a 38-m-long controlled environment chamber (Mayeux *et al.* 1993). Aerial growth of plants was enclosed in a transparent and tunnel-shaped polyethylene cover that was attached to the top of a 0.76-m-deep and 0.45-m-wide soil container. The plastic-lined soil container was partitioned into contiguous 0.6-m-long, water-tight compartments. Plants received no fertilizer and the approximately 12 m³ of fine sandy loam soil (Udic Paleustalfs; Huckabee *et al.* 1977) in the system was removed and mixed between experiments with *P. glandulosa* and *T. aestivum*. Soil contained 0.7% organic matter and 0.035% total N.

Photosynthesis by enclosed plants depleted the CO₂ and increased the ratio of ¹³CO₂ to ¹²CO₂ in air as it was moved by a blower from the air intake to the outlet of the chamber. A continuous gradient of [CO₂] from near 350 to 200 µmol mol⁻¹ was maintained during daylight (9 to 10 h daily) by automatically varying the rate of air flow through the chamber in response to changes in incident light and CO₂ depletion by enclosed plants. The [CO₂] was measured each minute with an infra-red gas analyser (Binos 67, Leybold-Haeraeus, Hanau, Germany) in air drawn from the chamber outlet and sequentially from five points spaced at 7.6-m intervals along the chamber. Blower speed was increased each night to exhaust respired CO₂.

Dry-bulb and dew-point air temperatures were regulated each 7.6 m along the chamber with chilled-water cooling coils and resistance heating elements to track these temperatures within the ventilated glasshouse. Dry-bulb and dew-point temperatures immediately before and after points of regulation were measured with fine-wire thermocouples and chilled-mirror hygrometers (DEW-10, General Eastern Instruments, Maine, USA). Vapour pressure deficit of air (VPD) was calculated from these records.

Soil water content in each of the closed 0.6-m-long soil compartments was measured weekly through the sides of the wooden soil containers with a TROXLER 3218 surface moisture gauge (Troxler Electronics, North Carolina, USA). An empirically determined relationship between the volume of water lost per compartment and the decline in gauge reading from that at 'field capacity' was used to determine evapotranspiration and to calculate water additions.

SPECIES AND CULTURE

Bromus tectorum

The C₃ annual grass, *B. tectorum*, was seeded on 5 February 1992 into 18 of the 0.6-m-long soil compartments along the chamber. Nine additional compartments each were planted on 1 April with three to five seeds of 'Gaspe' yellow flint *Zea mays*. The stable C isotope composition of *Z. mays* served as proxy for the ¹³C/¹²C of chamber air (Marino & McElroy 1991).

Soil in each evenly numbered of the consecutive compartments with *B. tectorum* and in all compartments with *Z. mays* was brought to 'field capacity' weekly (c. 20% volumetric water content; well-watered treatment). Soil in remaining compartments with *B. tectorum* was allowed to dry from 1 April until 6 May 1992 (droughted treatment). *Bromus tectorum* was thinned on 25 March and 6 May to 180 and 165 plants/soil compartment, respectively. Above-ground tissues or shoots (including crowns) of remaining *B. tectorum* and all *Z. mays* plants were removed on 18 May 1992. Two soil cores, each

45 mm in diameter, were composited per soil compartment with *B. tectorum* and roots were hand-separated from oven-dried (60 °C) soil. Kjeldahl nitrogen concentration ([N]) was measured on shoots and roots harvested from each compartment. Concentrations of ammonium- and nitrate-N in soil collected on 1 April to a depth of 0.3 m from each compartment with *B. tectorum* were measured following extraction with 2 N KCl.

Triticum aestivum

Yaqui 54 and Seri M82, day-neutral cultivars of spring wheat (*T. aestivum*), were planted on 12 February 1991 in 20 soil compartments along the 38-m chamber. Soil water content was restored to 'field capacity' weekly (well-watered treatment) in 10 soil compartments planted with each cultivar and until 3 April 1991 in remaining compartments, after which additional water was withheld for the last 50 days of the 100-day growing season (droughted treatment). Wheat was thinned on 5 and 18 March and 17 April to 240, 120 and 96 plants/soil compartment, respectively. Remaining above-ground material (including crowns) was completely harvested on 23 May after plants had matured and was divided into seed, chaff and remaining tissues (= shoots). Each tissue type was combined within individual compartments and [N] was determined by the Kjeldahl method.

Prosopis glandulosa

Individuals of the woody legume, *P. glandulosa*, were grown from seed planted 17 May 1989 as monocultures and in mixtures with four plants of the C₄ perennial grass *Schizachyrium scoparium* (Michx.) Nash. (little bluestem) in soil compartments along the chamber. Soil was kept near 'field capacity' during the first growing season from planting until 11 August 1989 and during the second growing season from 1 March 1990, when established plants broke dormancy, until 17 May 1990. At other times (August to December 1989 and May to October 1990), compartments received water only after soil moisture had declined to c. 65% of the volumetric content at field capacity. Senescent leaves of *P. glandulosa* were removed on 12 December 1989 from plants forced into dormancy by cold temperatures. Attached and fallen leaves and above-ground stems of *P. glandulosa* were completely harvested on 9 October 1990. Roots were hand-separated from soil cores from each compartment. Tap and lateral roots of *P. glandulosa* were completely excavated. Differences in stable C isotope composition between the C₃ *P. glandulosa* and C₄ *S. scoparium* were used to determine the root biomass of each in cores recovered from species mixtures (Polley, Johnson & Mayeux 1992b). Biomass and N masses (Kjeldahl method) from tissue collections in December 1989 and October 1990 were

summed to give cumulative biomass production and N accretion per *P. glandulosa* plant.

WATER- AND NITROGEN-USE EFFICIENCIES

The sum of above-ground biomass removed in thinning and whole-plant biomass (including roots) present at final harvest was divided by total evapotranspiration (sum of weekly water depletions) to calculate apparent WUE of *B. tectorum*. Apparent WUE of *T. aestivum* was calculated as above-ground biomass produced from 5 March to 23 May 1991 divided by water lost to evapotranspiration over the same period. Apparent WUE of *P. glandulosa* was cumulative biomass collected per monoculture divided by water lost to evapotranspiration.

Nitrogen-use efficiency (NUE) of the perennial *P. glandulosa* was calculated as the ratio of total biomass production to N accretion. This measure integrates production per unit plant N and N turnover. For annuals, NUE was the ratio of biomass to N in above-ground tissues (*T. aestivum*) or whole plants (*B. tectorum*) at final harvest. Values of NUE were divided by those for apparent WUE to determine the amount of water used per unit of plant N.

Tissue [N] often declines as plants grow, so single comparisons in time of biomass/N (NUE) from plants grown at different [CO₂] may be biased by effects of [CO₂] on plant growth and development (Coleman, McConnaughay & Bazzaz 1993). We used data on above-ground tissues from thinnings and final harvests of *B. tectorum* and *T. aestivum* to calculate for each species the slope of the linear regression of above-ground biomass on N at each [CO₂]. The slopes of these regressions indicate the amount by which above-ground biomass increased per unit of N invested and thus provide an index of plant NUE that is not biased by plant size against which to compare NUE calculated as biomass/N.

[CO₂] AND ENVIRONMENTAL VARIABLES

Environmental conditions during experiments with the C₃ annual, *T. aestivum*, and woody C₃ legume, *P. glandulosa*, have been detailed elsewhere (Polley *et al.* 1993b; Polley, Johnson & Mayeux 1994). Daytime [CO₂] during the experiment with *B. tectorum* was regulated at a minimum of 250 µmol mol⁻¹ beginning 3 weeks after planting and 200 µmol mol⁻¹ beginning 5 weeks post-planting. A continuous and linear gradient from a mean (±SE of daily means) 358 ± 1 to 207 ± 1 µmol mol⁻¹ was maintained for the final 8 weeks of the experiment, beginning 6 weeks post-planting. Mean daytime [CO₂] for plants in each 0.6-m-long soil compartment was estimated from the

linear regression of [CO₂] at the six locations routinely sampled on distance from the air intake. Chamber [CO₂] increased at night from a mean 372 $\mu\text{mol mol}^{-1}$ at the air intake to 392 $\mu\text{mol mol}^{-1}$ at the chamber outlet.

The mean dry-bulb temperature of air after regulation each 7.6 m along the chamber increased linearly from 20.3 °C on 1 March to 24.7 °C on 18 May 1992. The VPD of air after regulation did not vary temporally (\bar{x} of daily averages = 1.27 kPa, $n=34$ days). The dry-bulb temperature of air increased a mean 3.9 °C and air VPD decreased a mean 0.47 kPa between points of regulation each 7.6 m along the chamber. Night-time temperatures increased from 13.8 °C on 1 March to 20.2 °C on 18 May.

CARBON ISOTOPES

The $\delta^{13}\text{C}$ of air along the chamber was calculated as a function of daytime [CO₂] from the $\delta^{13}\text{C}$ of upper-canopy leaves of *Z. mays*, assuming a previously measured fractionation relative to air of 3.1‰ (Polley *et al.* 1993a). The $\delta^{13}\text{C}$ values of above-ground tissue of *B. tectorum* and those calculated for air were used to determine discrimination by *B. tectorum* against $^{13}\text{CO}_2$ (Δ). Temporally integrated values of leaf c_i/c_a (Farquhar, O'Leary & Berry 1982) and A/g or intrinsic WUE [net assimilation (A)/stomatal conductance to water (g)] then were calculated for *B. tectorum* assuming 29‰ as discrimination by ribulose-1,5-bisphosphate carboxylase against ^{13}C during CO₂ fixation (Roeske & O'Leary 1984). Leaf A/g of *T. aestivum* and *P. glandulosa* was derived similarly (Polley *et al.* 1993a, 1994).

For each species and cultivar, we calculated the A/g and NUE at 220 and 340 $\mu\text{mol mol}^{-1}$ from linear regressions of these parameters on [CO₂]. When the slope of linear regressions of NUE on [CO₂] did not differ significantly from zero, we used the mean NUE of plants grown across [CO₂] at both 220 and 340 $\mu\text{mol mol}^{-1}$. The ratio of A/g at 220 $\mu\text{mol mol}^{-1}$ to A/g at 340 $\mu\text{mol mol}^{-1}$ divided by the ratio of NUE at 220 $\mu\text{mol mol}^{-1}$ to NUE at 340 $\mu\text{mol mol}^{-1}$ provided an index of how this 120 $\mu\text{mol mol}^{-1}$ increase in [CO₂] affected the potential amount of water relative to plant N that each species and cultivar required to grow. Values of the index less than 1 indicate a greater relative increase in intrinsic WUE, A/g , than NUE with increasing [CO₂], or a decrease in the potential amount of water that plants required (independent of changes in leaf-to-air VPD) per unit N.

Results

NITROGEN CONCENTRATIONS AND USE EFFICIENCIES

Shoot [N] declined with time from planting in both cultivars of *T. aestivum*, indicating that growth was

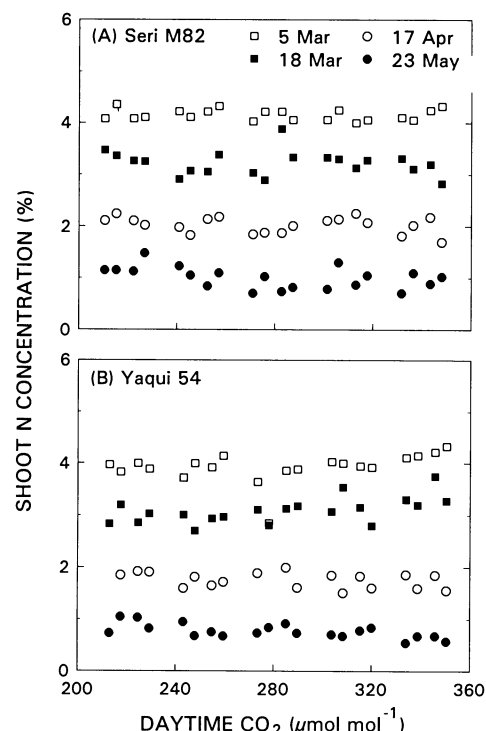


Fig. 1. The nitrogen concentration in shoots (above-ground tissues plus crowns) of well-watered and droughted plants of (A) Seri M82 ($n=20$) and (B) Yaqui 54 ($n=20$) cultivars of *Triticum aestivum* grown across a daytime gradient in [CO₂] from near 350 to 200 $\mu\text{mol mol}^{-1}$. Shoot [N] was measured on a subsample of plants removed from stands at each [CO₂] on 5 and 18 March and 17 April and on all remaining plants when *T. aestivum* was fully mature on 23 May 1991.

more rapid than N accretion (Fig. 1). The [N] of Yaqui 54 shoots harvested 18 March increased linearly from 2.8 to 3.3% with increasing [CO₂] ($r^2=0.35$, $P=0.006$, $n=20$; Polley *et al.* 1993b) but not until the first harvest did shoot [N] of Seri M82 differ with [CO₂]. Regressions of shoot [N] at final harvest on [CO₂] were coincident ($P>0.10$) for well-watered Seri M82 and Yaqui 54 and droughted Yaqui 54. Across these treatments, shoot [N] decreased linearly from 1.0 to 0.7% as [CO₂] rose from 210 to 350 $\mu\text{mol mol}^{-1}$ ($r^2=0.37$, $P=0.0004$), despite a linear increase in total N accretion from 1.3 to 2.5 g over the same range of [CO₂] ($r^2=0.39$, $P=0.0002$, $n=30$). The [N] of chaff from well-watered plants of each cultivar also declined linearly at higher [CO₂] but chaff [N] was 0.4% lower across [CO₂] in Seri M82 than Yaqui 54 (not shown). Chaff [N] was not related to [CO₂] in droughted plants (Seri M82, 1.1%, Yaqui 54, 1.2%). Seed [N] also did not change consistently with [CO₂] and averaged 2.8% in both cultivars across water treatments.

Despite the decrease in shoot [N] at higher CO₂, there was no consistent relationship of shoot NUE of either cultivar of wheat to [CO₂] (Table 1) nor did NUE differ greatly between water treatments (Fig. 2). Linear regressions of shoot biomass at three thinnings

plus the final harvest on shoot N content also revealed no consistent effects of $[\text{CO}_2]$ or water treatment on the amount of biomass produced per unit N invested ($\bar{x}=66.8 \text{ g g}^{-1}$ in Yaqui 54 and 61.7 g g^{-1} in Seri M82).

In contrast, the NUE of well-watered *B. tectorum* ($n=9$) increased linearly by almost 37% from 204 to $347 \mu\text{mol mol}^{-1}$ (Fig. 2) as the result of significant linear declines in both shoot (1.6 to 1.1% ; $r^2=0.54$, $P=0.03$) and root [N] at final harvest (Fig. 3). Root [N] declined linearly from 206 to $349 \mu\text{mol mol}^{-1}$ in droughted plants but shoot [N] and NUE did not vary consistently with $[\text{CO}_2]$ (Table 1, Fig. 2). Plant NUE was considerably lower across $[\text{CO}_2]$ in droughted than well-watered *B. tectorum* because droughted plants had higher shoot and root [N] than well-watered plants (Table 1). There was no significant effect of $[\text{CO}_2]$ on the ratio of root to shoot biomass or on total N accretion in either well-watered or droughted plants at harvest (not shown). Soil concentration of extractable nitrate and ammonium averaged 3.5 and $3.2 \mu\text{g g}^{-1}$, respectively, during the experiment and did not differ with position along the $[\text{CO}_2]$ gradient.

Estimates of the change in above-ground biomass per unit change in above-ground N, derived from linear regressions of biomass at two thinnings and the final harvest on N, qualitatively supported the trends in whole-plant NUE of *B. tectorum* at harvest. Above-ground biomass gained per unit N invested increased 17.6 g g^{-1} per $100 \mu\text{mol mol}^{-1}$ increase in $[\text{CO}_2]$ but was 24.6 g g^{-1} greater in well-watered than droughted plants across CO_2 levels.

We report elsewhere that the NUE of individually grown *P. glandulosa* ($n=6$) increased 40%, while total N accretion did not differ from 220 to $340 \mu\text{mol mol}^{-1}$ (Polley *et al.* 1994). The NUE of *P. glandulosa* grown in mixtures with the C_4 grass *S. scoparium* was not affected by $[\text{CO}_2]$ ($\bar{x}=66.3 \text{ g g}^{-1}$).

WATER-USE EFFICIENCIES

Apparent WUE of *T. aestivum* increased about $1 \text{ g biomass (l water)}^{-1}$ per $100 \mu\text{mol mol}^{-1}$ rise in $[\text{CO}_2]$ in all treatments, when two observations above $330 \mu\text{mol mol}^{-1}$ were excluded from the droughted treatment with Seri M82 (Fig. 4) but WUE was greater in droughted than well-watered plants of each cultivar (0.5 g l^{-1} for Yaqui 54, 0.7 g l^{-1} for Seri M82). The WUE of droughted *B. tectorum* was a curvilinear function of $[\text{CO}_2]$ ($r^2=0.89$, $P=0.001$, $n=9$) and increased from about 1.6 to near 3.0 g l^{-1} between 280 and $349 \mu\text{mol mol}^{-1}$. The linear correlation between apparent WUE of well-watered *B. tectorum* and $[\text{CO}_2]$ was only marginally significant ($P=0.065$).

There was a 67% increase in apparent WUE of individually grown *P. glandulosa* from 220 to $340 \mu\text{mol mol}^{-1}$ (Polley *et al.* 1994). We did not attempt to quantify the water that *P. glandulosa* used

Table 1. Mean N concentrations in roots and shoots of well-watered ($n=9$) and droughted ($n=9$) *Bromus tectorum* and mean nitrogen-use efficiencies of *B. tectorum* and two cultivars of *Triticum aestivum* ($n=10$ well-watered plus 10 droughted treatments for both Seri M82 and Yaqui 54) grown across daytime gradients in $[\text{CO}_2]$ from near 350 to $200 \mu\text{mol mol}^{-1}$. Variables that were statistically related to $[\text{CO}_2]$ are designated by * (see Figures 2 and 3)

Variable	Treatment	
	<i>Bromus tectorum</i>	
	Well-watered	Droughted
Root [N] (%)	0.7*	0.9*
Shoot [N] (%)	1.3*	2.1
Nitrogen-use efficiency [g biomass (g N) $^{-1}$]	87.4*	60.3
	<i>Triticum aestivum</i>	
	Seri M82	Yaqui 54
Nitrogen-use efficiency [g biomass (g N) $^{-1}$]	60.1	62.9

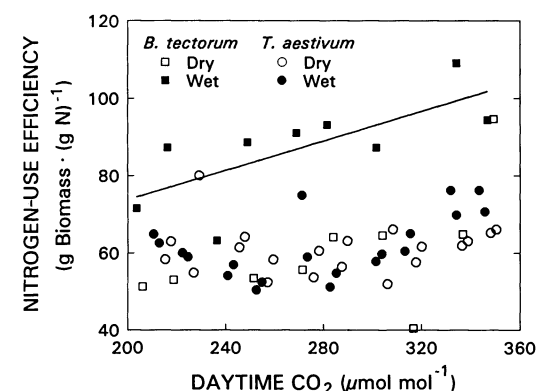


Fig. 2. Nitrogen-use efficiency (NUE), the ratio of biomass to plant N, of well-watered (Wet, $n=20$) and droughted (Dry, $n=20$) *Triticum aestivum* (Seri M82 and Yaqui 54 cultivars) and well-watered (Wet, $n=9$) and droughted (Dry, $n=9$) *Bromus tectorum*. The two species were grown in separate experiments across daytime gradients in $[\text{CO}_2]$ from near 350 to $200 \mu\text{mol mol}^{-1}$. The line is a linear regression fit for well-watered *B. tectorum* ($r^2=0.52$, $P=0.03$).

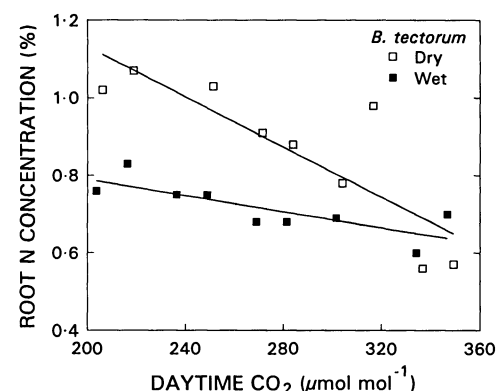


Fig. 3. The nitrogen concentration in roots of well-watered (Wet, $n=9$) and droughted (Dry, $n=9$) *Bromus tectorum* grown across a daytime gradient in $[\text{CO}_2]$ from near 350 to $200 \mu\text{mol mol}^{-1}$. Lines are linear regressions of root [N] on $[\text{CO}_2]$ ($r^2=0.63$, $P=0.01$ for Wet and $r^2=0.71$, $P=0.005$ for Dry).

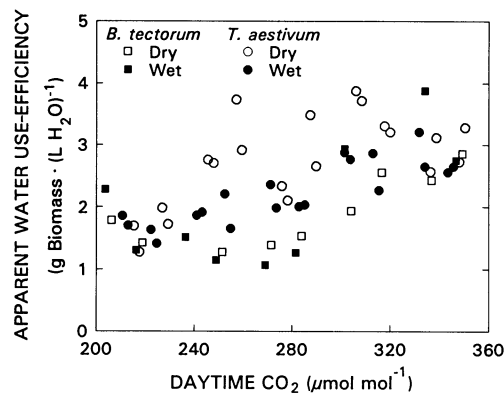


Fig. 4. Apparent water-use efficiency (WUE), biomass produced divided by water lost to evapotranspiration, of well-watered (Wet, $n = 20$) and droughted (Dry, $n = 20$) *Triticum aestivum* (Seri M82 and Yaqui 54 cultivars) and well-watered (Wet, $n = 9$) and droughted (Dry, $n = 9$) *Bromus tectorum*. The two species were grown in separate experiments across daytime gradients in [CO₂] from near 350 to 200 $\mu\text{mol mol}^{-1}$.

when grown in the same experiment with the C₄ grass *S. scoparium*. The A/g calculated from leaf isotope analyses of *P. glandulosa*, however, did not differ between plants that were grown in monocultures and mixtures with the grass and increased by nearly the same relative amount as did [CO₂] (44% with 55% increase in CO₂).

RELATIVE REQUIREMENTS FOR WATER AND NITROGEN

Averaged across [CO₂], the amount of water that wheat lost to evapotranspiration per unit plant N, NUE divided by apparent WUE, was higher for Yaqui 54 (28.41 g^{-1}) than Seri M82 (24.61 g^{-1}) and in well-watered (28.71 g^{-1}) than droughted treatments (24.31 g^{-1}). Across cultivars and water regimes, the ratio of water lost to plant N was a curvilinear function of [CO₂] and decreased from 40.71 g^{-1} at 210 $\mu\text{mol mol}^{-1}$ to 24.01 g^{-1} or below when [CO₂] rose above 270 $\mu\text{mol mol}^{-1}$ (Fig. 5).

Conversely, the amount of water lost from *B. tectorum* stands per unit plant N did not change consistently with [CO₂] in either the well-watered or droughted treatments. The trend toward higher NUE with increasing [CO₂], particularly in well-watered plants, offset any increase in apparent WUE. Mean values of water loss per unit N were virtually identical for *B. tectorum* grown in five well-watered compartments (33.11 g^{-1}) and the nine compartments with a drought treatment (33.21 g^{-1}). Water loss/N was higher for well-watered *B. tectorum* in the remaining four compartments. This may have reflected high evaporative losses but water loss/N in these compartments was not apparently related to differences in air VPD or temperature along the chamber.

There was also no systematic change with [CO₂] in water loss per unit N of individually grown *P. glandulosa*. Because A/g increased and the NUE of *P. glandulosa* grown with *S. scoparium* did not change at higher [CO₂], water use per unit plant N of mixture-grown *P. glandulosa* apparently declined as [CO₂] rose.

CARBON ISOTOPES

The $\delta^{13}\text{C}$ values of leaves from the C₄ *Z. mays* and of above-ground tissues from the C₃ *B. tectorum* increased linearly (became more enriched in ^{13}C) as [CO₂] decreased (Fig. 6). Leaf $\delta^{13}\text{C}$ values were c. 1–2‰ higher in droughted than well-watered *B. tectorum* across [CO₂] but in neither treatment was isotopic discrimination of *B. tectorum* altered by [CO₂]. In *B. tectorum*, as in *T. aestivum* (Polley *et al.* 1993a) and *P. glandulosa* (Polley *et al.* 1994), A/g increased by about the same relative amount as did [CO₂] (77% in well-watered and 66% in droughted plants with the 64% increase in [CO₂] from 210 to 345 $\mu\text{mol mol}^{-1}$; Fig. 7).

The ratio of A/g at 220 $\mu\text{mol mol}^{-1}$ to A/g at 340 $\mu\text{mol mol}^{-1}$ divided by the ratio of NUE at 220 $\mu\text{mol mol}^{-1}$ to NUE at 340 $\mu\text{mol mol}^{-1}$ ranged

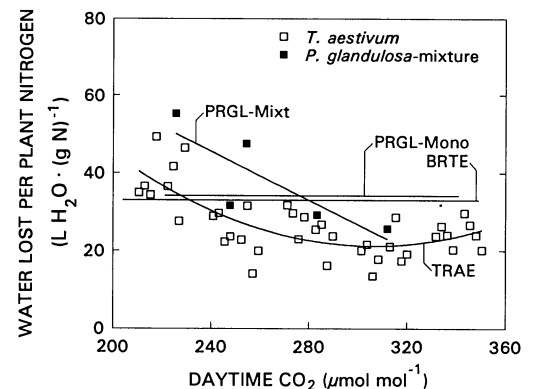


Fig. 5. Water lost to evapotranspiration per unit of plant nitrogen [plant nitrogen-use efficiency (NUE)/apparent water-use efficiency (WUE)] for C₃ plants grown across daytime gradients in [CO₂] from near 350 to 200 $\mu\text{mol mol}^{-1}$. Data are from separate experiments with *Triticum aestivum*, *Bromus tectorum* and *Prosopis glandulosa*. Where water lost per unit plant N did not vary significantly with [CO₂] (*P. glandulosa* grown in monocultures, PRGL-Mono, $n = 6$, and well-watered plus droughted *B. tectorum*, BRTE, $n = 8$), the mean value across the [CO₂] gradient is indicated by a horizontal line. Values of apparent WUE for *P. glandulosa* grown in mixture with the C₄ grass *S. scoparium* ($n = 5$) were calculated with carbon isotope-based values of leaf intrinsic water-use efficiency (A/g) from the linear relationship of A/g to WUE of individually grown *P. glandulosa* from the same experiment. Observed NUE was divided by calculated WUE and resulting values were fit with a linear regression on [CO₂] (PRGL-Mixt, $r^2 = 0.67$). Data for well-watered and droughted Seri M82 and Yaqui 54 cultivars of *T. aestivum* were fit with a quadratic regression on [CO₂] (TRA, $r^2 = 0.53$, $P < 0.0001$, $n = 40$).

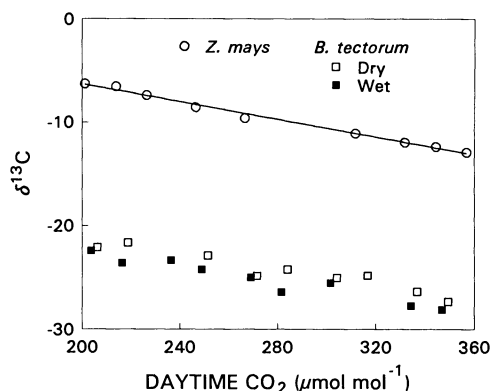


Fig. 6. Stable C isotope composition ($\delta^{13}\text{C}$) of upper leaves from *Zea mays* and above-ground tissues from well-watered (Wet) and droughted (Dry) *Bromus tectorum* plants grown along a gradient in daytime $[\text{CO}_2]$ ($n = 9$ in each case). The line is a linear regression fit of $\delta^{13}\text{C}$ values of *Z. mays* on daytime $[\text{CO}_2]$ ($r^2 = 0.99$, $P < 0.0001$).

from 0.64 for droughted *B. tectorum* to 0.96 for individually grown *P. glandulosa*. This index was 0.71 for well-watered plants of both Yaqui 54 and Seri M82, 0.70 for *P. glandulosa* grown with *S. scoparium* and 0.78 for well-watered *B. tectorum*. Raising $[\text{CO}_2]$ from 220 to 340 $\mu\text{mol mol}^{-1}$ thus decreased the potential amount of water required per unit N for all but individually grown *P. glandulosa*, in which a 44% increase in A/g from 220 to 340 $\mu\text{mol mol}^{-1}$ was matched by a comparable relative increase in plant NUE (Polley *et al.* 1994).

Discussion

Plant use of water relative to N may have decreased, in some cases substantially, as atmospheric CO_2 rose from glacial to present concentrations but observed responses varied with species and growth conditions. If the past rise in $[\text{CO}_2]$ changed plant resource requirements relative to availabilities, it could have altered ecosystem function and plant species composition independently of other direct effects of CO_2 on vegetation. How greatly and consistently plant use of water relative to N changed among C_3 species during the past 18 000 years depend both on species differences in NUE and WUE at a single $[\text{CO}_2]$ and the extent to which plant and environmental variables mediated the requirements of vegetation for these resources as $[\text{CO}_2]$ rose.

NITROGEN-USE EFFICIENCY

Of the two components of the ratio of water used to plant N, WUE and NUE, the latter was least responsive to $[\text{CO}_2]$ and varied most among species and treatments in this study. Plant NUE did not change with $[\text{CO}_2]$ and was similar for *P. glandulosa* grown with the C_4 *S. scoparium*, droughted *B. tectorum* and two cultivars of *T. aestivum*. Conversely, NUE rose

with $[\text{CO}_2]$ in well-watered *B. tectorum* (Fig. 2) because of decreases in shoot and root [N] and in *P. glandulosa* grown in monoculture because of both increased biomass distribution to roots and lower root [N] (Polley *et al.* 1994).

Similar changes in components of plant NUE often occur when $[\text{CO}_2]$ is raised above the current level. Several reports noted that tissue [N] declined when plants were grown at elevated $[\text{CO}_2]$ (Hocking & Meyer 1985; Norby, O'Neill & Luxmoore 1986a; Curtis, Drake & Whigham 1989; Norby *et al.* 1992). Furthermore, the [N] of whole plants is decreased and NUE is increased by greater plant investment in tissues that have inherently low [N]. At elevated $[\text{CO}_2]$, this often is manifested as an increase in the ratio of plant roots to shoots (Norby, Pastor & Melillo 1986b; Larigauderie, Hilbert & Oechel 1988; Norby & O'Neill 1991) and a decrease in the ratio of leaf area or mass to plant biomass (Norby & O'Neill 1991; Norby *et al.* 1992). A decrease in N turnover also would increase NUE of perennials (Berendse & Aerts 1987). No evidence exists, however, that higher $[\text{CO}_2]$ increased N recycling in *P. glandulosa* (Polley *et al.* 1994) or other C_3 perennials (Norby *et al.* 1986b; Curtis *et al.* 1989).

The latitude for variation in NUE among plants in nature may be more limited than suggested by some studies. Each of the means by which plants modify NUE, and thus NUE itself (Aber *et al.* 1991), potentially is a function of N availability (Shaver & Melillo 1984). The NUE of individual species or vegetation as a whole, when calculated as the inverse of [N] in one or more tissues, usually decreases as N availability increases (Vitousek 1982; Pastor *et al.* 1984; Birk & Vitousek 1986). Mixing and replacing the soil in our chamber between experiments likely altered N mineralization rates and thus may have contributed to the

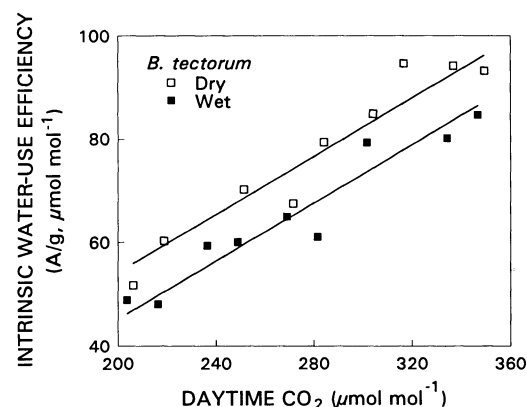


Fig. 7. Intrinsic water-use efficiencies [net assimilation (A)/stomatal conductance to water (g)] derived from $\delta^{13}\text{C}$ values of above-ground tissues of well-watered (Wet, $n = 9$) and droughted (Dry, $n = 9$) *Bromus tectorum* grown along a gradient in daytime $[\text{CO}_2]$. The slopes of linear regressions of A/g on $[\text{CO}_2]$ did not differ significantly between water treatments ($P > 0.25$), so data for well-watered and droughted plants are fit with parallel lines.

differences in NUE observed among species. Availability of N in many ecosystems, however, is constrained because N in excess of plant and microbial demand may be lost. Furthermore, it often is impractical to measure N turnover in all tissues, so most estimates of NUE do not include effects of N turnover integrated over whole plants (Berendse & Aerts 1987). Recent research indicates that when species differences in N turnover and distribution among tissues are considered, whole-plant NUE is more similar among perennial plants than formerly thought (Berendse & Aerts 1987; Aerts 1990; Shaver & Chapin 1991).

Consequently, a large portion of any increase in NUE that C₃ plants realized as [CO₂] rose probably was related to the amount of N that was diverted from tissues and processes involved in C assimilation to structures with inherently low [N]. While whole-plant [N] often declines at higher [CO₂], it is not clear whether this simply reflects dilution of available N by more rapidly growing plants (Coleman *et al.* 1993) or represents reallocation of N from C assimilation. In either case, there is a maximum C:N ratio that can be tolerated by any one species and the decline in plant [N], and associated increase in NUE, cannot continue indefinitely (Shaver *et al.* 1992). The high rates at which some non-photosynthetic tissues turnover also may constrain any CO₂-induced increase in plant NUE. Plants sometimes invest more of total biomass and, presumably, N in fine roots when [CO₂] is raised (Norby *et al.* 1992). The high turnover rates of fine roots from which limited retranslocation of N may occur (Aerts 1990) may greatly reduce the efficacy of allocation changes in increasing NUE.

WATER-USE EFFICIENCY

Our evapotranspiration-based calculations of apparent WUE increased with higher [CO₂] in all species and treatments, though less consistently than did isotope-based values of A/g. The effect of increasing CO₂ on apparent WUE may have been underestimated if respiration of the plants grown at low day-time [CO₂] was inhibited by the higher night-time [CO₂] to which they were exposed. Unlike apparent WUE which also was influenced by soil evaporation and possibly by repeated gradients in VPD along the chamber, A/g of the three species studied increased by roughly the same relative amount as did [CO₂]. Several factors, including evaporative demand of the atmosphere, the fraction of fixed C that plants respire, and plant transpiration in the absence of photosynthesis affect the correlation between A/g and plant biomass/transpiration. Each of these factors, of course, may vary in nature. Evaporative demand, for example, is a function of atmospheric humidity and temperature and, during the last 18 000 years, was influenced by apparently correlated changes in temperature and moisture (Barnola *et al.* 1987; Adams &

Woodward 1992). Transpiration also is influenced by [CO₂]-caused shifts in stomatal conductance which modify leaf temperature (Morison & Gifford 1984). In addition, C₃ plants exhibit substantial, genetically based variation in WUE that may be retained across environments (Ehleringer & Cooper 1988; Hubick & Farquhar 1989; Ehleringer *et al.* 1990; DeLucia & Schlesinger 1991). Both environmental factors and genetic differences should thus have caused realized WUE to vary among species at a given [CO₂] and possibly promoted differential responses to increasing [CO₂].

RELATIVE REQUIREMENTS FOR WATER AND NITROGEN

Any effect of the past increase in [CO₂] on the amount of water relative to N that C₃ plants used evidently was sensitive to environmental conditions and species traits. Of the two components contributing to water use/plant N, WUE may have varied most among species and across past [CO₂] because of the feedbacks that exist between transpiration and evaporative demand of the atmosphere and between stomatal conductance and leaf temperature. We have argued elsewhere, however, that because photosynthetic rates rise sharply from glacial to present [CO₂], a large fraction of the past increase in WUE of C₃ plants was realized as biomass production, independently of changes in stomatal conductance or transpiration (Polley *et al.* 1993a). Trends in our data thus suggest that at least some C₃ plants now require less transpired water per unit absorbed N to grow than they did in the past when [CO₂] was lower. This may have been particularly true in most natural settings, and especially in late successional vegetation, where N availability and potential changes in plant [N] and NUE presumably were limited.

In the extreme case where NUE has not changed with increasing [CO₂], some plants may now require only half as much water per unit N as they did during the last ice age, 18 000 years ago. One current view is that N limitation will constrain or entirely negate any positive growth response of plants to elevated [CO₂] (Shaver *et al.* 1992). The assumption is that N availability to plants, largely via mineralization, will not increase, at least in the short term of years to decades. If this assumption is extended over the last 18 000 years to times when [CO₂] was lower and plant use of water relative to N perhaps was higher, then C₃ plants at one time may have been relatively more water-limited than today. Plant response to past changes in [CO₂] then would have been less constrained by N availability than will be the immediate response to future, elevated [CO₂].

The assumption that N availability has not changed appreciably during the past 18 000 years likely is too simplistic because cycles of water and N are intimately associated. In particular, ecosystem N accu-

mulation is linked to water availability and plant WUE through the influence of these factors on biomass accumulation (Vitousek & Reiners 1975). Aber *et al.* (1991) demonstrated that litter quality, patterns of plant allocation and N deposition affect the rate at which N-cycling increases but not the ultimate level that is reached. As long as net N input to an ecosystem is positive, the amount of N that is retained and cycled will continue to increase with biomass accumulation until plant growth is limited by the availability of other resources or the ability of plants to translate resources into biomass (resource-use efficiencies).

Potential production and, by extension, ecosystem N accumulation should have increased in ecosystems that were not subjected to frequent losses of N as $[\text{CO}_2]$ and plant WUE increased. The several thousand years that intervened between the increase in $[\text{CO}_2]$ to its preindustrial level and the 27% increase in the last 200 years appear more than adequate to support increased N cycling and plant growth even in ecosystems that initially were N-limited. About one-half of the total increase in atmospheric $[\text{CO}_2]$ since glaciation has occurred in the last 200 years. This change in $[\text{CO}_2]$ may have been too rapid to promote substantial N accumulation. Nevertheless, ecosystems in which production is now limited by N presumably were less severely N-limited 200 years ago when plant consumption of water relative to N may have been as much as 27% greater than today. Even if the historical increase in $[\text{CO}_2]$ has not elicited greater C_3 production in N-limited systems, it may, by preferentially increasing plant WUE over NUE, have caused substantial changes in ecosystem water yield and the vertical distribution of soil water. These changes, in turn, could have influenced growth form composition in some systems (Knoop & Walker 1985). Competitive balance and plant-species distributions through time may also have been sensitive to interspecific variation in the amount of water required per unit N and the response of resource requirements to changing $[\text{CO}_2]$ (Tilman 1993).

Conclusions

Increasing $[\text{CO}_2]$ differentially alters the efficiencies with which some plants use water and N. Potential consequences include local shifts in species composition and vegetation structure, altered water and N cycles, and greater production in C_3 -dominated ecosystems. These largely indirect or secondary effects of CO_2 could conceivably have exerted as great an influence on ecosystem composition and function as the more direct effects of $[\text{CO}_2]$ on C assimilation.

These data and the literature demonstrate, however, that plants may vary substantially in the extent to which $[\text{CO}_2]$ altered their use of water relative to N. A

large fraction of this variation apparently is driven by the environment but the possibility that use of water relative to N differed consistently among various growth forms or functional groups of C_3 species has not been explored.

Some of the evidence discussed above, however, suggests that WUE, NUE and the relative amounts of water and N that C_3 plants use in common environments may be conservative enough to allow first-order predictions of plant growth from resource availabilities. Aber *et al.* (1991), for example, successfully modelled N-mineralization rates in mature forest stands by treating WUE as a constant within species and NUE as a function of N availability. A number of ecosystem processes are sensitive to interactions among vegetation and water and N availabilities (Pastor & Post 1986, 1988). Perhaps a first step in evaluating the potential application of $[\text{CO}_2]$ -driven changes in plant resource requirements to questions of vegetation dynamics and ecosystem function is to determine how greatly and consistently plant requirements for water and N vary across relevant CO_2 levels and among C_3 species with different growth forms.

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